

## AMENDMENTS TO THE SPECIFICATION

*Please amend page 3, lines 13-18 as follows:*

Another preferred embodiment of the present invention the mono-enzyme- or the bi-enzyme-system is crosslinked into an osmium redox polymer. The osmium-based redox polymer is preferably (PVI<sub>13</sub>-dmeOs) of poly-(1-vinyl-imidazole), complexed with [Os(4,4'-dimethylbipyridine)<sub>2</sub>Cl]<sup>+/+2</sup> [Os-(4,4'-dimethylbipyridine)<sub>2</sub>Cl]<sup>+/+2</sup>, and a crosslinking agent such as poly-(ethyleneglycol)-diglycidyl-ether (PEGDGE).

*Please amend page 3, lines 28-34 as follows:*

Amine oxidase represents a class of enzymes with a ubiquitous distribution in mammals, plants and micro-organisms. However, the structure, selectivity and biological functions are very different, depending on the isolation source. Grass-pea amine oxidase, ~~for~~ for instance, is a copper-containing amino oxidase, which besides the metal ions also contains an organic cofactor with a quinoide structure (topa-quinone) in its catalytic site.

*Please amend page 3, lines 36-38 as follows:*

In methods, where an amine oxidase is used, the enzyme is converting the amine to the corresponding aldehyde, with NH<sub>3</sub> and H<sub>2</sub>O<sub>2</sub> release, according to the following reaction I:

*Please amend page 4, lines 22-34 as follows:*

According to the bi-enzymatic approach of the invention, the bi-enzyme electrode configuration is based on the enzyme amine oxidase (AO), from grass pea, and horseradish peroxidase (HRP) on a solid graphite electrode. The bi-enzymatic system is working at a potential where biases are minimal. The bi-enzyme electrodes were prepared either by simply adsorbing the two enzymes on the electrode surface (DET) or by crosslinking them into a redox polymer (MET). In the latter case, the highly permeable and stable redox hydrogel is formed of a poly(1-vinylimidazole) complexed with  $[\text{Os}(\text{4},\text{4}'\text{-dimethylbipyridine})_2\text{Cl}]^{+/+2}$  ( $\text{PV}_{13}\text{-dmeOs}$ )  $[\text{Os-(4,4}'\text{-dimethylbipyridine})_2\text{Cl}]^{+/+2}$  ( $\text{PVI}_{13}\text{-dmeOs}$ ), and crosslinked to the enzymes by a crosslinking agent, e.g., e.g. poly-(ethyleneglycol)-diglycidyl-ether (PEGDGE).

*Please amend page 5, lines 17-25 as follows:*

~~Then the active form of the enzyme being recovered by oxidation of the organic cofactor in presence of molecular oxygen, see mechanism II.~~ The hydrogen peroxide formed during the first reaction is subsequently reduced to water by the action of peroxidase. The native form of the second enzyme is re-made either by direct reduction of its heme cofactor on the electrode surface or by receiving electrons from a mediator, maintained in it's reduced form by the potential applied on the graphite electrode (50 mV vs. Ag/AgCl).

*Please amend page 6, lines 10-12 as follows:*

The structure of the redox polymer  $[\text{Os}(\text{4},\text{4}'\text{-dimethylbipyridine})_2\text{Cl}]$   $[\text{Os-(4,4}'\text{-dimethylbipyridine})_2\text{Cl}]$  complexed to poly(1-vinyl-imidazole)] is shown in the following formula:

*Please amend page 7, lines 27-34 as follows:*

*B7*  
Type II electrodes: electrodes were prepared by cross-linking 6  $\mu$ l of a mixture formed of AO (stock solution 20 mg/ml in PB), HRP (stock 10 mg/ml in PB) with an osmium redox hydrogel. The osmium redox hydrogel consisted of PV<sub>13</sub>-dmeOS PVI<sub>13</sub>-dmeOS (stock 10 mg/ml in PB) and PEGDGE (5 mg/ml freshly prepared and used within 15 min). The bi-enzyme cross-linked into the redox hydrogel was placed on the top of the graphite electrode in different ratios in % by weight (w/w).

*Please amend page 8, lines 11-14 as follows:*

*B8*  
Type III c - in the first step, a drop of HRP solution (6  $\mu$ l) was placed ~~an~~ on the top of the electrode, and after its drying, a second layer containing 6  $\mu$ l of a premixed solution of AO, PVI13-dmeOs PVI<sub>13</sub>-dmeOs, and PEGDGE was added.

Please amend Table I on page 10 as follows:

TABLE I

Type of electrode (w/w)	Analyte	$K_m^{app}$ ( $\mu M$ )	$I_{max}$ ( $\mu A$ )	S ( $mA/Mcm^2$ )	C (%)	DL ( $\mu M$ )	
AO 87%	Histamine	279±16	1.03±0.02	50.57±0.82	19.0	0.16	
HRP 13%	Putrescine	153±15	1.96±0.06	175.48±1.40	66.2	0.06	
	$H_2O_2$	93±3	1.80±0.21	265.13±1.65	-	-	
AO 80%	Histamine	332±17	<u>1.34±0.03</u> <u>1.03±0.03</u>	55.28±0.76	16.6	0.20	
HRP 20%	Putrescine	228±15	<u>3.01±0.07</u>	180.84±0.95	54.7	0.07	
	$H_2O_2$	112±8	2.07±0.06	330.23±1.02	-	-	
AO 67%	Histamine	370±22	1.30±0.03	48.13±0.14	14.7	0.25	
HRP 33%	Putrescine	240±15	3.10±0.01	176.94±0.87	54.2	<u>0.70</u> <u>0.07</u>	
	$H_2O_2$	153±6	3.64±0.04	325.90±0.56	-	-	
AO 50%	Histamine	437±43	1.22±0.04	38.24±1.42	12.7	0.33	
HRP 50%	Putrescine	268±23	3.05±0.10	155.90±1.26	52.0	0.08	
	$H_2O_2$	175±8	3.83±0.05	299.80±0.65	-	-	
AO 40%	Histamine	441±23	1.16±0.02	36.03±0.75	10.9	0.34	
HRP 60%	Putrescine	276±22	3.69±0.06	183.14±1.11	55.7	0.13	
	$H_2O_2$	206±3	4.94±0.03	328.50±0.22	-	-	
AO 33%	Histamine	479±41	1.37±0.10	39.18±1.54	12.2	0.41	
HRP 67%	Putrescine	287±12	3.84±0.06	183.28±0.61	57.0	0.08	
	$H_2O_2$	211±18	4.95±0.15	321.36±1.24	-	-	

Please amend page 12, lines 12-23 as follows:

b10  
The influence of various components of the redox hydrogel on the biosensor characteristics is shown in Table IV. The increasing  $K_m^{app}$  in the presence of both PVI<sub>13</sub>-dmeOs and PEGDGE demonstrated that the diffusion of the substrate was limited. This was because of the barrier formed by the mediator and/or cross-linking agent (rigidity of the redox hydrogel) on the surface of the electrode, which also resulted in an increased linear dynamic range. On the other hand, in the presence of crosslinked redox polycationic mediator (PVI<sub>13</sub>-dmeOs), the  $I_{max}$  value was 100% increased suggesting that the final reduction step of the topa cofactor on the electrode surface is the rate-limiting step in the absence of the methyldiator mediator?

Please amend page 13, lines 1-3 as follows:

b11  
In Table IV summarizes the response characteristics of different AO biosensors. The AO, PVI<sub>13</sub>-dmeOs and PEGDGE concentrations were 5 mg/ml, 2 mg/ml and 0.5 0.5 mg/ml, respectively.

Please amend Table IV on page 13 as follows:

TABLE IV

Type of electrode	$K_m^{app}$ ( $\mu M$ )	$I_{max}$ (nA)	S (mA/Mcm <sup>2</sup> )	DL ( $\mu M$ )	DR ( $\mu M$ )
AO	375±34	164±6.5	5.99±0.09	2.7	10-100
AO+PEGDGE	755±38	185±5.0	3.35±0.05	4.5	10-150
AO+PVI <sub>13</sub> -dmeOs AO+PV1 <sub>13</sub> -dmeOs	770±14	235±2.4	4.18±0.02	3.7	10-150
AO+PV1 <sub>13</sub> -dmeOS+ PEGDGE	730±33	360±8.0	6.76±0.05	2.2	10-200

*Please amend page 15, lines 14-17 as follows:*

The substrates are histamine His, cystamine Cys, tyramine Tyr, spermidine Spr, ~~etylenediamine~~  
*B12* ~~ethylenediamine~~ EDA, agmatine Agm, putrescine Put, cadaverine Cad, Z-Ab-Z-1,4-diamino-2-  
butene and E-Ab-E1 ,4-diamino-2-butene.